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Belay, Difabachew K.; Huckaba, Randy M.; Ramirez, Axel M.; Rodrigues, Jose C. V.; and Foster, John E., "Insecticidal Control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Transmitting Carlavirus on Soybeans and Detection of the Virus in Alternate Hosts" (2012).

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Published in *Crop Protection* 35 (May 2012), pp. 53–57; doi: 10.1016/j.cropro.2011.12.020

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Submitted February 15, 2011; revised November 1, 2011; accepted December 31, 2011; published online February 10, 2012.

Insecticidal Control of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Transmitting Carlavirus on Soybeans and Detection of the Virus in Alternate Hosts

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Abstract

A Carlavirus transmitted by *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important disease of soybean nurseries in Puerto Rico causing substantial germplasm losses. Insecticide bioassay experiments were conducted at Dow AgroSciences (DAS) Research Station, Santa Isabel, Puerto Rico, either by spraying insecticides on *B. tabaci* infested soybean leaves or introducing *B. tabaci* adults onto insecticide-sprayed soybean leaves. Moreover, host plants were surveyed to detect the virus in overwintering hosts that serve as a source of inoculums. The direct spray experiment showed that Nuprid 2F (Imidacloprid), Capture 2 EC (Bifenthrin), Thionex (Endosulfan), Lannate LV (Methomyl), and Dimethoate gave good level (> 80%) of control of *B. tabaci*. However, in the second set of the bioassay test, the residual activity of all of the insecticides was generally low. Except Thionex, all

the insecticides resulted in less than 50% mortality on adult *B. tabaci* that were introduced 24 h after spray. The host plant survey showed that 8 out of the 18 commonly occurring plant species gave positive result to the Enzyme-linked immunosorbent assay (ELISA) test.

Keywords: *Bemisia tabaci*, Carlavirus, insecticides, bioassay, ELISA, soybean

1. Introduction

Whiteflies (WFs) (Hemiptera: Aleyrodidae) are important polyphagous pests of several plant species throughout the world that feed on at least 60 different families of plants (Byrne and Bellows, 1991). WFs are small insects with piercing-sucking mouth parts in which both immature and adult stages feed on the underside of leaves. The life cycle of WFs includes egg, four nymphal stages, and the adult stage. According to Byrne and Bellows (1991), most species of WFs develop from egg to adult within 25–50 days under field conditions, and there could be at least six generations per year. In areas like Dow AgroSciences Research Station, Santa Isabel, Puerto Rico, where different types of vegetables and wild hosts are available throughout the year that can serve as overwintering and over-summering hosts of WFs, chemical control is the major option. Hence, selecting more effective insecticides and using them in rotation/combination is necessary. One hundred fourteen virus species are transmitted by WFs, and *B. tabaci* transmits 111 of these species (Jones, 2004).

In Puerto Rico, where many of the seed companies including Dow AgroSciences (DAS) grow their winter soybean (*Glycine max* L.) nurseries to advance breeding programs, the nurseries are being attacked by Carlavirus that causes stem and leaf necrosis, stunting, shoot wilting, and death of the soybean plants. The virus is believed to be transmitted by WFs in a nonpersistent manner which can be acquired and transmitted within a few minutes or hours of feeding (Duffus, 1987). Previous studies showed that the whitefly population in Puerto Rico is biotype B of *B. tabaci* (Rodrigues et al., 2008; Brown et al., 1995; Frohlich et al., 1999; J. C. V. Rodrigues, unpublished data). The same viral disease transmitted by WFs on soybeans has been reported from Brazil and the disease was caused by a whitefly-born Carlavirus which is related to Cowpea mild mottle virus (CpMMV) (Almeida et al., 2005).

The source of the virus for spreading into crops could be found among plants in the crop, which could be either weeds or cultivated plants (internal sources) or outside borders, i.e., virus-infected wild and cultivated plants (external sources) (Harris, 1983). Usually wild plants, volunteers, and perennials may serve as overwintering or over-summering hosts of the virus (Bos, 1981). In Santa Isabel, Puerto Rico, where DAS Research Station is located, several species of alternate hosts of WFs are available year round which could serve as a source of Carlavirus infection into soybean fields. The objectives of the present study were to (1) screen insecticides that can effectively control *B. tabaci* which transmits the Carlavirus and (2) investigate the source of infestation of the WFs and the virus itself on soybean fields.

2. Materials and methods

2.1. Insecticide screening for the control of *B. tabaci*

Insecticide bioassay experiments were conducted using field-collected *B. tabaci* populations under laboratory conditions at 25°C and 65–70% relative humidity. The bioassay was conducted in two different ways: insecticides were sprayed on *B. tabaci*-infested soybean leaves or *B. tabaci* adults were introduced onto insecticide-sprayed soybean leaves. The WF species used in our study was identified at the University of Puerto Rico, Department of Virology, San Juan, and it belongs to *B. tabaci* biotype B. Moreover, previous studies on WF populations have shown that the Puerto Rico WF species is *B. tabaci* (Rodrigues et al., 2008; Brown et al., 1995).

2.1.1. Direct spray of insecticides onto *B. tabaci*-infested soybean leaves

Adult *B. tabaci* were collected from tomato plants using an aspirator made from small diameter plastic hoses and plastic cups. The bioassay was conducted using small rectangular plastic boxes whose lids have a ventilation hole for aeration, which was covered by a nylon mesh. A single healthy soybean leaf was placed in each box (dish) and moist cotton was attached to the petiole to prevent desiccation of the leaves. Ten adult WFs collected from tomato fields were introduced into each cup, and insecticide formulations (Table 1) prepared based on manufacturers' recommended rates were sprayed onto the leaves while the *B. tabaci* were feeding. Four replications per treatment were used in a completely randomized design (CRD). Mortality of *B. tabaci* was assessed at 3, 6, and 24 h after spray. For each sampling date, there was a control treatment that was sprayed with an equal amount of distilled water.

Table 1. List of insecticides screened in the insecticide bioassay experiments against *Bemisia tabaci* in soybeans

Treatment number	Trade name	Active ingredient	Rate	Unit	ml/l
1	Nuprid 2F	Imidacloprid	24	oz/a	3.75
2	Capture 2 EC	Bifenthrin	6.4	oz/a	1.00
3	Orthene 97	Acephate	8	oz/a	1.25
4	Thionex	Endosulfan	3	Pts/a	7.50
5	Lannate LV	Methomyl	1.5	Pts/a	3.75
6	Mycotrol ES	<i>Beauveria bassiana</i>	4	oz/a	0.62
7	Ultrafine oil	Paraffinic oil 99.8%	0.5	gl/50gl	2.64
8	Safe-T-side	Petroleum oil 80%	0.5	gl/50gl	2.64
9	Dimethoate 4E	Dimethoate	1	Pts/a	2.50
10	Control	—	—	—	—

2.1.2. Exposure of *B. tabaci* to soybean leaves sprayed with insecticides

In order to assess the residual effects of the insecticides, spray formulations were prepared according to the company's rate of application. Fresh soybean leaves whose petioles were covered with moist cotton to prevent desiccation were sprayed with insecticides and left

on a laboratory bench. After 3, 6, or 24 h of spray, the leaves were placed into plastic boxes as described above, and ten adult *B. tabaci* were introduced into each cup onto the leaves. Mortality of *B. tabaci* was assessed after 3 and 24 h of adult introduction with a destructive sampling. The treatments were replicated four times in CRD.

2.2. Data analysis

All mortality values were corrected for natural mortality using Abbot's formula (Abbott, 1925) as: $CM\% = [(C_A - T_A)/C_A] \times 100$, where: CM = Corrected mortality, C_A = Control alive, and T_A = Treatment alive. Data were analyzed using the Proc GLM procedure of SAS (SAS Institute, 1999), and whenever ANOVA showed significant treatment effects, individual treatment means were separated using Student Newman Keuls test (SNK) procedure. Data distribution was checked using the box plot method in SAS and when necessary, percent data were arcsine square root transformed before statistical analysis. The significance level was set to $P = 0.05$.

2.3. Study on source of infection of Carlavirus on soybeans

The objective of this experiment was to identify source of the WFs (*B. tabaci*) and the virus itself that infect the soybean nurseries at DAS in Puerto Rico. Commonly grown cultivated and wild hosts were surveyed in DAS and nearby farms. Leaf samples were collected in plastic zip lock bags on blue ice. Samples were collected from plant species that were observed infested with *B. tabaci*. A total of 18 plant species (Table 4) were surveyed and weeds were identified using a handbook prepared for identification of common weeds of Puerto Rico (Torres and Laracuente, 2002). Detection of Carlavirus in the samples was done by enzyme-linked immunosorbent assay (ELISA) using a commercial ELISA kit with polyclonal antibody for Cowpea Mild Mottle Virus (CPMMV) (DSMZ Company, Germany). ELISA procedures were conducted according to the manufacturer's recommendation provided with the kit. Samples were replicated 4–8 times and each sample was repeated two times.

3. Results

3.1. Insecticide screening for *B. tabaci* control

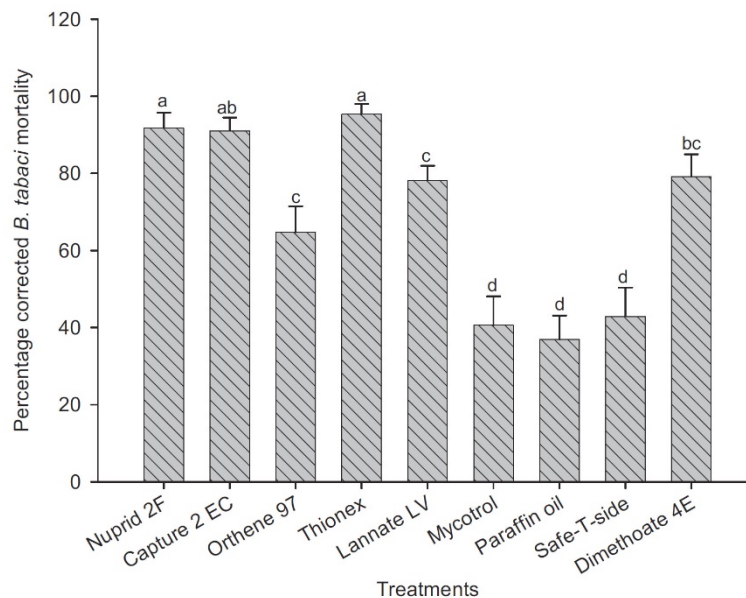
B. tabaci mortality varied with length of exposure (feeding) time after insecticide application ($F_{6,78} = 1.80$, $P = 0.0498$). Hence, treatments were compared at specific mortality assessment time. After 3 h of insecticide application, higher *B. tabaci* mortality was recorded from Nuprid 2F, Capture 2 EC, Thionex, and Lannate LV (Table 2). Except for Paraffin Oil and Mycotrol, all insecticides applied onto *B. tabaci* caused significantly higher mortality than the untreated control. Six hours after spray, Nuprid 2F, Capture 2 EC, Thionex, Lannate LV, and Dimethoate caused higher *B. tabaci* mortality than the other insecticides. Similarly, after 24 h of insecticide application, Nuprid 2F, Capture 2 EC, Thionex, and Dimethoate resulted in a significantly higher *B. tabaci* mortality (Table 2).

Table 2. Mean (\pm SE) percent corrected mortality (CM) of *Bemisia tabaci* in an insecticide-screening experiment after 3, 6, or 24 h of insecticide application

Treatments	3 h	6 h	24 h
Nuprid 2F	96.3 \pm 3.6a	78.8 \pm 8.7a	100.0 \pm 0.0a
Capture 2 EC	88.9 \pm 7.0a	93.2 \pm 6.7a	90.8 \pm 5.6a
Orthene 97	53.1 \pm 14.9bc	70.4 \pm 14.1ab	70.6 \pm 3.4b
Thionex	88.9 \pm 7.0a	96.9 \pm 3.0a	100.0 \pm 0a
Lannate LV	76.7 \pm 4.5ab	87.2 \pm 7.3a	70.6 \pm 5.9b
Mycotrol	33.6 \pm 18.17c	40.4 \pm 15.3bc	47.8 \pm 3.8c
Paraffin oil	33.3 \pm 12.8c	27.2 \pm 8.5c	50.0 \pm 10.0c
Safe-T-side	46.9 \pm 10.0bc	14.5 \pm 5.7c	67.1 \pm 3.8b
Dimethoate 4E	66.9 \pm 15.1ab	83.3 \pm 6.7a	87.1 \pm 4.8a

Means within a column followed by the same letter(s) are not statistically different from each other (SNK, $P = 0.05$).

Overall, Nuprid 2F, Capture 2 EC, Thionex, Lannate, and Dimethoate gave better (> 80%) control of *B. tabaci* s than the rest of the treatments (Fig. 1). Moreover, average *B. tabaci* mortality caused by all treatments is lower at 3 h after insecticide application than after 24 h (Fig. 2).

**Figure 1.** Average percent corrected mortality of *Bemisia tabaci* by different insecticides evaluated after 3, 6, and 24 h of spray. Bars followed by the same letter are not significantly different from each other (SNK, $P = 0.05$).

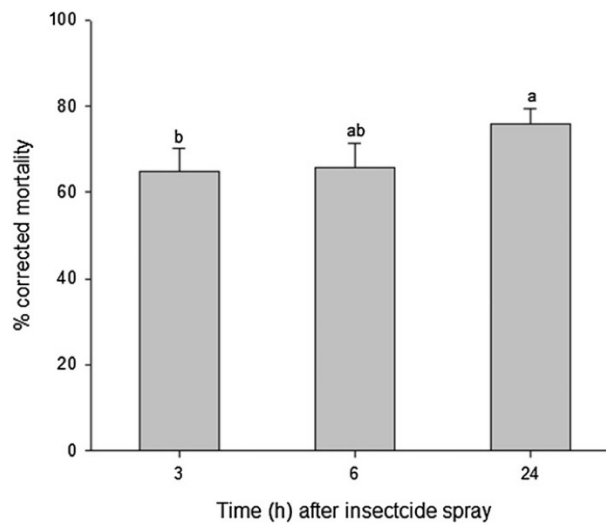


Figure 2. Mean percent corrected mortality of *Bemisia tabaci* after 3, 6, or 24 h of insecticides spray. Bars followed by the same letter are not significantly different from each other (SNK, $P = 0.05$).

3.2. Insecticides residual activity test

In this experiment, there was a significant three-way interaction between treatments, time after leaf spray and duration of *B. tabaci* exposure to the insecticide-treated soybean leaves ($F_{10,184} = 2.96$, $P = 0.0018$). Therefore, treatments were compared at the same residual period and length of exposure time. Generally, the level of mortality from the residual effects of insecticides was lower compared to the direct spray on the *B. tabaci* feeding on the soybean leaves (Table 3).

After 3 h of exposure of *B. tabaci* to insecticide-sprayed soybean leaves before 3 h, the highest (74.9%) and the lowest (35.2%) mean percent *B. tabaci* mortality was obtained from Nuprid 2F and Orthene 97, respectively. However, after 24 h of exposure for the same residual period, there was a 100% mortality in Thionex-treated leaves, and this effect of Thionex was consistent for both 3, 6, and 24 h time after leaf spray (Table 3).

Table 3. Mean (\pm SE) percent corrected mortality of *Bemisia tabaci* after 3, 6, or 24 h of insecticide application onto soybean leaves and 3 and 24 h of exposure of *B. tabaci* adults to the treated leaves

Treatments	3 h after leaf spray		6 h after leaf spray		24 h after leaf spray	
	3 h% CM	24 h% CM	3 h% CM	24 h% CM	3 h% CM	24 h% CM
Nuprid 2F	66.8 \pm 9.0a	51.4 \pm 6.0bcd	73.5 \pm 7.4a	67.5 \pm 9.0bc	12.9 \pm 6.8bc	44.4 \pm 5.0b
Capture 2 EC	23.5 \pm 9.5b	59.8 \pm 0.0bcd	51.0 \pm 3.4ab	79.0 \pm 5.5bc	47.6 \pm 7.9a	19.8 \pm 5.0bc
Orthene 97	15.6 \pm 7.3b	37.5 \pm 2.2d	26.5 \pm 8.4b	62.8 \pm 22.3bc	13.4 \pm 9.2bc	47.5 \pm 7.7b
Thionex	47.1 \pm 13.3ab	100.0 \pm 0.0a	71.2 \pm 6.3a	100.0 \pm 0.0a	35.2 \pm 4.7a	100.0 \pm 0.0a
Lannate LV	47.3 \pm 13.5ab	77.7 \pm 22.3ab	54.2 \pm 8.5ab	77.9 \pm 9.6ab	21.7 \pm 10.6abc	2.2 \pm 2.2d
Mycotrol	52.0 \pm 27.7ab	75.9 \pm 24.1abc	31.3 \pm 10.8ab	38.7 \pm 4.3cd	3.8 \pm 3.0c	33.5 \pm 16.0b
Paraffin oil	38.0 \pm 11.9ab	38.6 \pm 6.0dc	25.15 \pm 9.6b	12.2 \pm 7.0d	7.7 \pm 5.2c	10.4 \pm 7.7cd
Safe-T-side	28.6 \pm 3.2ab	21.4 \pm 10.7d	18.3 \pm 11.7b	45.4 \pm 11.1bc	22.6 \pm 6.9abc	21.1 \pm 5.1bc
Dimethoate 4E	52.8 \pm 4.3ab	34.4 \pm 13.1d	42.0 \pm 13.0ab	51.6 \pm 12.2bc	19.8 \pm 7.1bc	36.3 \pm 11.4b

Means within a column followed by the same letter(s) are not statistically different from each other (SNK, $P = 0.05$). CM = Corrected mortality

In the 6 h residual period, Nuprid 2F, Capture 2 EC, Lannate LV, and Thionex caused a similar level of higher mortality than other treatments after 3 h of exposure. However, after 24 h of exposure, Thionex gave the highest level of *B. tabaci* mortality than others (Tables 2 and 3). For the 24 h residual period, 3 h of exposure of *B. tabaci* to the insecticide-treated leaves generally resulted in a lower level of mortality in all insecticide treatments. But with the extended time of exposure (24 h), Thionex resulted in a significantly higher (100%) *B. tabaci* mortality than the rest of the treatments (Tables 2 and 3). Looking at overall residual effect of the insecticides on *B. tabaci* mortality, Thionex is the most effective insecticide against WFs followed by Nuprid 2F, Capture 2 EC, and Lannate LV (Fig. 3). The overall residual effect of the insecticides is better after 3–6 h of spray than after 24 h (Fig. 4).

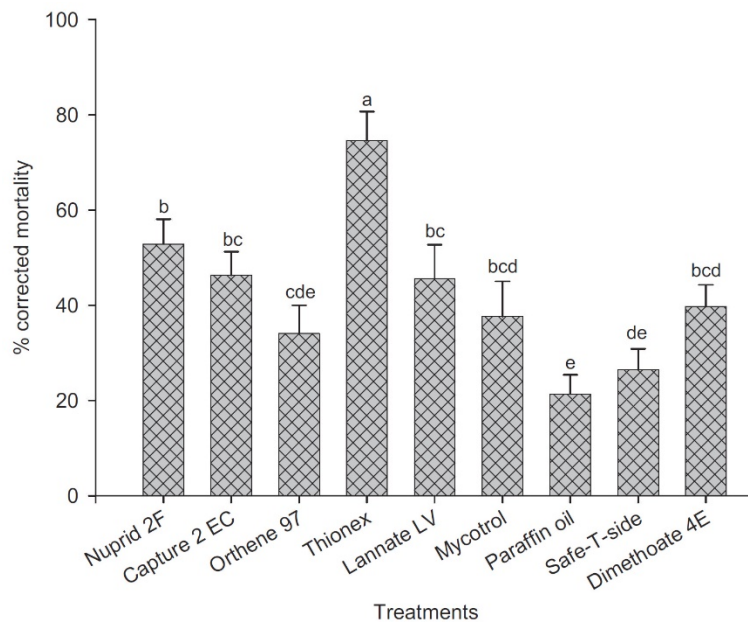


Figure 3. Overall mean percent corrected mortality of *Bemisia tabaci* due to residual effect of different insecticides. Means followed by the same letter(s) are not different from each other (NNK, $P = 0.05$).

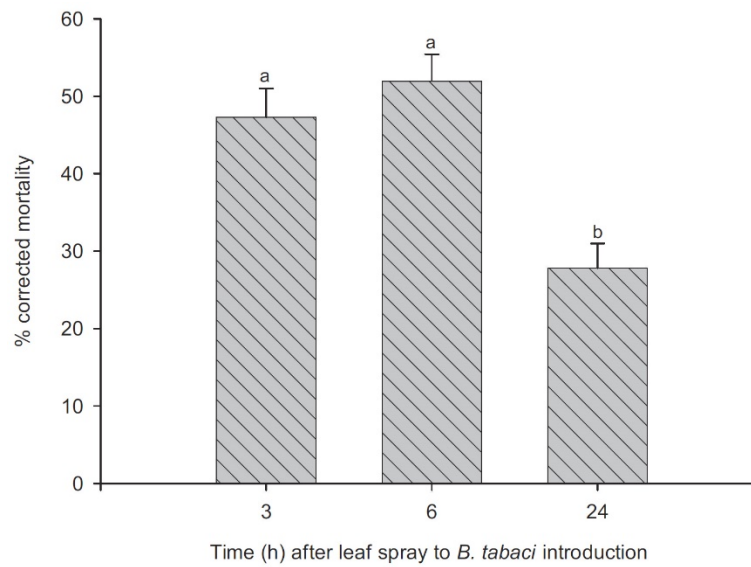


Figure 4. Percent corrected mortality of *Bemisia tabaci* exposed to soybean leaves after 3, 6, or 24 h of insecticide spray.

3.3. Study on source of infection of Carlavirus on soybeans

The ELISA results showed that 2 out of 5 cultivated plant species and 6 out of 13 uncultivated species (Table 4) tested gave positive results for the Carlavirus. Percent Carlavirus infection varied 25–50% (Table 4). However, none of the plant species that showed positive reaction to the ELISA test expressed visible symptoms of the disease. These host plants are grown both within the crop fields and outside the crop (external sources).

Table 4. Cultivated and uncultivated host plants screened for Carlavirus detection at Dow Agro-Sciences Research Station, Santa Isabel, Puerto Rico, and ELISA results, May 2010

Scientific name	ELISA result	Status	# plants tested	% infection
<i>Vigna unguiculata</i> L.	Positive	Cover crop	8	25
<i>Glycine max</i> (L.) Merr.	Positive	Cultivated	8	25
<i>Lycopersicon esculentum</i> L.	Negative	Cultivated	4	0
<i>Cucurbita</i> sp.	Negative	Cultivated	4	0
<i>Ipomoea batatas</i> (L.) Lam.	Negative	Cultivated	4	0
<i>Ludwigia octovalvis</i> (Jacq.)	Positive	Weed	4	25
<i>Solanum americanum</i> Mill.	Positive	Weed	4	25
<i>Trianthema portulacastrum</i> L.	Positive	Weed	4	25
<i>Ipomea</i> sp.	Positive	Weed	4	25
<i>Boerhavia erecta</i> L.	Positive	Weed	4	25
<i>Argemone mexicana</i> L.	Positive	Weed	4	50
<i>Euphorbia heterophylla</i> L.	Negative	Weed	4	0
<i>Amaranthus dubius</i> Mart.	Negative	Weed	4	0
<i>Malvaceae</i> sp.	Negative	Weed	4	0
<i>Datura stramonium</i> L.	Negative	Weed	4	0
<i>Cleome gynandra</i> L.	Negative	Weed	4	0
<i>Kallstromia maxima</i> L.	Negative	Weed	4	0
<i>Macroptium lathyroides</i> (L.) Urban	Negative	Weed		0

4. Discussion and conclusions

Insecticides can provide a convenient and economically feasible method of pest control and are important to global food and fiber production (Eichers, 1981), if the appropriate rate and type of insecticide is used. In the present study we have screened insecticides that can control *B. tabaci* which transmit Carlavirus in soybeans. The efficacy of the insecticides tested in this study was variable in controlling the WFs. Similarly Perring et al. (1999) mentioned that one of the problems in using insecticides is that their effectiveness against vectors of plant pathogens is variable. In Puerto Rico where a *B. tabaci*-borne Carlavirus is attacking soybean nurseries, our first task was to select insecticides that can best control the vector and identify host plants that serve as a source of inocula. This in turn will help to reduce the disease spread, as fewer insects acquire virus because the inoculum source plants will be reduced (eliminated) and also those WFs that can acquire the virus will be prevented from inoculating healthy plants as they are treated with insecticides.

Although some of the insecticides used in this experiment provided adequate control of *B. tabaci* when directly sprayed on *B. tabaci*-infested leaves, their residual activity was generally low. Except Thionex, no insecticide was able to provide even 50% control after 24 h of spray (Table 3). The short residual period of the insecticides tested shows that frequent applications are necessary; otherwise, incoming vectors could spread virus between application intervals. However, even in the case of contact insecticides with a longer residual period, covering new growing leaves (parts) will be impossible, which may allow virus transmission on unprotected foliage.

Thionex, which is an organochlorine insecticide, is characterized by low solubility in water and is more stable in the environment (Hill and Waller, 1982) and this long residual effect is observed in our experiments. However, the slow degradation may lead to gradual accumulation of the insecticide and long-term contamination of the environment. Dimethoate, which has shown a good level of WF control in the direct spray experiment, seems to have a low residual effect. Many of the organophosphate insecticides including Dimethoate are usually nonpersistent and have less residual effect (Edwards, 1987). For the successful control of diseases resulting from nonpersistently transmitted viruses like Carlavirus, insecticides must kill the vector rapidly, repel it, or modify vector behavior to prevent probing (Broadbent, 1957; Heinrichs, 1979). According to Perring and Farrar (1993), synthetic pyrethroids that repel or cause rapid knockdown or mortality of vectors prior to virus inoculation are the most successful class of insecticides used to reduce virus spread. However, the insecticides used in the present study with a knockdown effect including the pyrethroid, Capture 2 EC, showed very short persistence and low level of *B. tabaci* control even one day after spray.

As we have seen from the ELISA results, not all host plants infested with *B. tabaci* have the Carlavirus. This indicates that all WFs that infest soybean plants may not carry the virus unless they visited other infected plants before they arrive onto the soybean plants. Hence, understanding which WF populations may carry the virus helps to schedule chemical control programs. Moreover, the ELISA results revealed that the plant species that serve as source of inocula for the spread of the virus into soybean fields occur within and outside the crop. Harris (1983) also mentioned that the source of the virus for spreading into crops could be found among plants in the crop, which could be either weeds or cultivated plants (internal sources) or outside borders, i.e., virus-infected wild and cultivated plants (external sources). Hence, management of insect-vectored viral disease with a broad host plant species needs cooperation of growers in the area to control the vectors and alternate hosts. However, growers may become reluctant to incur personal expenses when the perceived benefits are broadly dispersed. It is very rare that a grower's best economic interest is to help reduce his neighbors' virus problems (Perring et al., 1999).

In areas like Santa Isabel, Puerto Rico, where a variety of horticultural crops and uncultivated plant species are grown year round that allow carryover of *B. tabaci* infestation, identifying susceptible stages of soybean plants to Carlavirus infection will help to support insecticide spray decisions to control the WFs. In a preliminary experiment, we have observed that soybean plants infested with *B. tabaci* at first trifoliate stage showed the disease symptom compared to those infested at first true leaf or second trifoliate stages.

In general, the bioassay study indicated that foliar application of Nuprid 2 F, Capture 2 EC, Thionex, and Dimethoate can provide good control of *B. tabaci*. Managing host plants, particularly weeds, that serve as a source of inoculums for the WFs as well as the virus and application of insecticides based on identified susceptible stages of the soybean plants is important to manage the Carlavirus problem.

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